DOING RESEARCH IN THE INTRAMURAL PROGRAM OF THE NATIONAL INSTITUTES OF HEALTH

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Introduction

In trying to describe the contributions of intramural research at the National Institutes of Health (NIH) to international collaboration, I thought it appropriate to show the role of many foreign visitors in our research on catecholamine neurotransmitters. This personal tale illustrating how research is done at NIH doubtless could be repeated in different forms by many others who also have benefited from the splendid cooperative arrangements at NIH.

I joined NIH in 1950, just as the Intramural Program of the National Heart Institute was started under the direction of James Shannon. While working in the National Heart Institute, I obtained a Ph.D. at George Washington University. After getting a Ph.D. in 1955 at the age of 42, I was invited by the National Institute of Mental Health (NIMH) to set up a section on pharmacology. I accepted with anticipation and some apprehension. My impression of neuroscience was that it was mainly concerned with electrophysiology and brain anatomy and involved the use of complicated electronic equipment. I believed that an investigator in neuroscience had to be an especially gifted experimentalist and theorist. Ed Evarts, who was then head of the laboratory in which my section belonged, assured me that it was not necessary to work on the brain or mental illness; I could study anything I pleased.

I began a research problem with which I felt comfortable and that might be related to the mission of the NIMH. Several years earlier, I had worked on the metabolism of drugs. At the NIMH my initial problem was concerned with the metabolism of LSD and narcotic drugs [1, 2]. One of the advantages of doing research in Bethesda is the variety of

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physical and intellectual resources available. Bob Bowman of the National Heart Institute was in the process of building a new kind of spectrophotometer. He was kind enough to let me use one of his experimental models, which enabled me to develop an exquisitely sensitive assay for LSD and to study its metabolism and distribution in the brain and other tissues. I also turned my attention to investigating the metabolism of narcotic drugs. An enzyme in liver microsomes was found that N-demethylated narcotic drugs [2]. This enzyme was profoundly affected during the development of tolerance to narcotic drugs [3]. On the basis of these findings I proposed a theory of tolerance that stimulated a great deal of critical reaction, mostly negative. This theory predicted the presence of opiate receptors and their down-regulation after continous interaction with narcotic drugs.

The Fate of Noradrenaline at the Nerve Terminal

I felt somewhat guilty about not working directly with the nervous system, although the administrators of the NIMH were supportive of the research I was doing. An opportunity to begin investigation on a problem more relevant to the mission of the NIMH occurred in an unexpected way. In 1956, Seymour Kety, who was then the head of my laboratory, reported at a seminar on a preliminary observation made by two Canadian psychiatrists, A. Hoffer and H. Osmond, that struck my interest. They found that when adrenaline was left exposed to the air it was converted to adrenochrome. When adrenochrome was ingested it produced hallucinations. Osmond and Hoffer then proposed that schizophrenia might be produced by an abnormal metabolism of adrenaline to adrenochrome. In view of these observations, I thought it would be a good idea to study the metabolism of adrenaline. In 1956, it was believed that adrenaline and other catecholamines were metabolized and inactivated by monoamine oxidase. I spent 4 frustrating months trying to find an enzyme that converts adrenaline to adrenochrome.

In March 1957, I received the *Federation Proceedings*, and I noticed an abstract by Armstrong and McMillan [4]. They reported that in patients with adrenaline-forming tumors (pheochromocytoma), large amounts of 3-methoxy-4-hydroxymandelic acid were excreted in the urine. From the structure of this compound it appeared to me that it might be a metabolic product of either adrenaline or noradrenaline by deamination and O-methylation. The possibility that catecholamines could be O-methylated was intriguing and could be examined experimentally.

The most likely methyl donor for the methylation of a hydroxy group was S-adenosylmethionine. I then incubated adrenaline with a rat liver homogenate, ATP, and methionine. The latter compounds should be

converted to S-adenosylmethionine in the liver. I was delighted to observe that adrenaline was rapidly metabolized under these conditions. When either ATP or methionine was omitted, there was little metabolism of the catecholamine. Similar results were obtained when S-adenosylmethionine was used. These were crucial experiments and demonstrated a new pathway for the metabolism of catecholamines by O-methylation. The most likely site of methylation would be on the hydroxyl group in a position meta to the side chain, to form O-methyl adrenaline. I called Bernard Witkop, a bioorganic chemist at NIH, and asked if he or his associates could synthesize the O-methylated metabolite of adrenaline. Three days later, Sino Senoh, a visiting scientist from Osaka, synthesized meta O-methyl adrenaline, which we named metanephrine. This is an example of the exceptional resources available at NIH. Metanephrine was identified after incubating liver with S-adenosylmethionine and adrenaline. This compound was also found to be present in rat urine, and amounts increased after adrenaline injection. The responsible enzyme was isolated and purified and shown to O-methylate other catecholamines, such as noradrenaline, dopamine, and dopa [5]. Because of these properties, this enzyme was named catechol-O-methytransferase (COMT). O-Methylated catecholamines normetanephrine and 3-methoxytyramine were soon found to be present in the brain [6]. I felt that I was a neuroscientist at last. As a result of the discovery of COMT, the pathway for the metabolism of catecholamines was described as involving monoamine oxidase and COMT.

It was believed in 1957 that neurotransmitters were inactivated by the enzyme monoamine oxidase. After inhibiting both monoamine oxidase and COMT, however, the physiological actions of noradrenaline rapidly disappeared after the injection of noradrenaline. If it was not enzymes, what was the mechanism for terminating the actions of catecholamines? The answer to this important question came with the help of two visiting scientists to my laboratory, Gordon Whitby from Cambridge University and Georg Hertting from the University of Vienna.

When Whitby arrived in Bethesda in 1959, I suggested that he examine the tissue distribution of [^{3}H]noradrenaline in cats. Dr. Kety commissioned New England Nuclear Corporation to synthesize [^{3}H]noradrenaline so that he could examine the metabolism of this amine in schizophrenic and normal subjects. He gave me a few microcuries of [^{3}H]noradrenaline for the animal studies. Parenthetically, I note that Kety and co-workers found no difference in the metabolism of [^{3}H]noradrenaline between schizophrenics and normal subjects. We observed that after the injection of [^{3}H]noradrenaline in cats, the catecholamine persisted unchanged in tissues rich in the sympathetic nerves, heart, spleen, salivary gland, and adrenal medulla long after its physiological effects had dissipated [7]. It became apparent that [^{3}H]noradrenaline

was selectively retained in sympathetic nerves and glands that concentrate catecholamines.

George Hertting, who came as a visiting scientist while these experiments were going on, suggested an elegant approach to establish that the site of uptake of catecholamine neurotransmitters was the sympathetic nerves. First, the superior cervical ganglia of a cat was removed unilaterally, causing the sympathetic nerves in the salivary gland and eye muscles to degenerate on one side only. A week later, [^{3}H]noradrenaline was injected and its concentration determined in structures innervated by nerves arising from the superior cervical ganglia. There was little or no uptake of [^{3}H]noradrenaline in denervated structures as compared to innervated tissue [8]. This experiment provided strong evidence for a new mechanism for the inactivation of a neurotransmitter—reuptake into sympathetic nerve terminals. We were then in a position to label the sympathetic nerve selectively by injecting [^{3}H]noradrenaline and then to examine the physiological disposition and metabolism of this neurotransmitter during and after nerve stimulation. In a series of experiments, Hertting [9], Sune Rosell [10], a visiting scientist from the Karolinska Institute, and I showed that noradrenaline is taken up in the sympathetic nerves and released on stimulation. In another experiment in collaboration with Keith Richardson, a British anatomist who was spending some time at NIH, we directly demonstrated by radioautographic studies and electron microscopy that [^{3}H]noradrenaline is taken up and stored in vesicles of sympathetic nerves [11]. It was with the collaboration of a number of visiting scientists that we were able to describe a model for the fate of the neurotransmitter noradrenaline in sympathetic nerve terminals [12] that has stood the test of time. This model made it possible to explain the actions of psychoactive drugs such as amphetamine, cocaine, and antidepressants.

With the observation that antidepressant drugs block the reuptake of noradrenaline in peripheral sympathetic nerves, it appeared that this process could explain the mechanism of action of drugs that relieve mental depression. It was a visiting postdoctoral fellow, Jacques Glowinski from Paris, who made it possible to examine this problem. Glowinski devised a technique to introduce [^{3}H]noradrenaline into the brain by injecting it into the lateral ventricle. In collaboration with a visiting scientist from England, Hans Weil-Malherbe, we had previously shown that [^{3}H]noradrenaline does not cross the blood-brain barrier. To study the action of antidepressant drugs on catecholamines, it was necessary to inject [^{3}H]noradrenaline directly into the brain. We found that radioactive noradrenaline injected into the lateral ventricle was distributed in different areas of the brain in about the same proportions as were the endogenous amines. After labeling the noradrenergic neurones with [^{3}H]noradrenaline, Glowinski and I found that pretreat-

ment of rats with clinically effective antidepressant tricyclic drugs blocked the neuronal uptake of [^{3}H]noradrenaline [13]. Tricyclic compounds related in structure to antidepressant drugs but with little or no clinical therapeutic activity did not block the reuptake of [^{3}H]noradrenaline. This, together with the observation that monoamine oxidase inhibitors have antidepressant actions and that reserpine, a depletor of noradrenaline and other amines, sometimes causes depression, led to the formulation of the catecholamine hypothesis of depression [14]. Although this hypothesis has not entirely held up, it has provided a productive framework for new experimental approaches to the study of depression.

Regulation of Catecholamines

Catecholamine neurotransmitters in sympathetic nerves are in a state of flux, continually being synthesized, released, metabolized, and recaptured. In spite of these dynamic changes, the concentration of catecholamines in tissues remains at a constant level. This is due to a variety of adaptive mechanisms that change the biosynthesis, release, and response of catecholamines. One such mechanism was discovered with the help of Hans Thoenen, a visiting scientist from Basel. Thoenen asked me if he could spend a sabbatical year in my laboratory. Before that, Thoenen and his co-workers had found that the injection of 6-hydroxydopamine destroyed sympathetic nerve terminals. I wrote to Thoenen that we would be happy to have him join our laboratory, especially if he brought some 6-hydroxydopamine with him.

When Thoenen arrived in Bethesda, the first experiment we did was to study the localization of the catecholamine biosynthetic enzyme, tyrosine hydroxylase. In this experiment we exploited the ability of 6-hydroxydopamine to destroy sympathetic nerve terminals selectively. Tyrosine hydroxylase disappeared after the destruction of sympathetic nerves with 6-hydroxydopamine, indicating the selective localization of tyrosine hydroxylase in noradrenergic nerves [15]. An unexpected finding was a marked increase in tyrosine hydroxylase in the adrenal medulla after treatment with 6-hydroxydopamine [15]. It was known that 6-hydroxydopamine also causes an increased firing of sympathetic nerves, and we speculated that the increased tyrosine hydroxylase was due to increased sympathetic nerve activity. We then injected a number of drugs that caused increased sympathetic nerve activity; these drugs also increased tyrosine hydroxylase activity in the adrenal medulla and sympathetic ganglia. When nerves to the adrenal gland were cut, none of the drugs that caused heightened sympathetic nerve activity could elevate tyrosine hydroxylase, indicating that induction of this enzyme was a transsynaptic event [16]. Subsequent experiments showed that

increased nerve activity induced the synthesis of new tyrosine hydroxylase molecules. Similar results were obtained with another biosynthetic catecholamine enzyme, dopamine-β-hydroxylase.

The National Institutes of Health have also served as a productive training ground for young Ph.D.'s and M.D.'s who wish to pursue careers in biomedical research. I found that these postdocs create an open atmosphere in the lab, and free exchange of ideas has made it possible to try new approaches to the solution of problems. An example of the value of the interaction with postdoctoral fellows is the experimental demonstration in which the relationship between the adrenal cortex and medulla was demonstrated. It has been known that the ratio of adrenaline and noradrenaline in the adrenal medulla is dependent on the extent to which the adrenal cortex surrounds the medulla. In those species in which the adrenal cortex is in close juxtaposition to the medulla, the main catecholamine is the methylated catecholamine, adrenaline, while in species where the adrenal cortex is separated from the medulla, the principle catecholamine is noradrenaline. In an exchange of ideas about the role of the cortex in regulating adrenaline formation, Richard Wurtman, a postdoctoral fellow in my laboratory, proposed an experiment to determine how the adrenal cortex could regulate adrenaline formation. He removed the pituitary from a rat and then measured the adrenaline-forming enzyme, phenylethanolamine-N-methyltransferase (PNMT). The pituitary secretes ACTH, which then stimulates the adrenal cortex to synthesize glucocorticoids. The ablation of the pituitary gland resulted in a profound decrease in PNMT [17]. The administration of either glucocorticoids or ACTH to these rats restored enzyme activity. This experiment clearly demonstrated that glucocorticoids from the adrenal cortex regulate the adrenaline-forming enzyme.

These are a few examples of the value of the visiting scientist and postdoctoral program in promoting high-quality research in the Intramural Research Program at NIH. More than 60 postdoctoral fellows and visiting scientists have spent time in my laboratory, and more than 50 percent of these postdocs were found overseas. With one or two exceptions, they all went on to highly productive careers in research. They all told me that the years that they spent in Bethesda had an important influence on their subsequent careers in biomedical research.

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